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Research Article

Deciphering the cytotoxic activity of *annona squamosa* iron oxide nanoparticles against selective cancer cell line

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ABSTRACT

In this present investigation, using hydroethanolic extract of leaves of *Annona squamosal,* iron oxide nanoparticle was synthesised at 60^0 c temperature and detected by UV–Visible spectrophotometer between 300 to 700 nm. The structure of nanoparticles was observed by SEM and FT-IR was performed to know the major functional groups. In anticancer assay, Fe_3O_4 NPs showed significant cytotoxicity on HepG2 and melanoma A375 cell lines. At the same time, Fe_3O_4 NPs showed zero toxicity on normal liver cell line. From our results, we could observe, this as a eco-friendly and nontoxic bio-reductant for the synthesis of Iron oxide nanoparticle with potential for cancer therapy.

Keywords: *Annona squamosal;* nanoparticles; liver cancer; melanoma; iron oxide nanoparticles; MTT assay; cytotoxicity assay.

INTRODUCTION

For the past few centuries we have been extensively using various medicinal plants as drugs for wide range of diseases traditionally such as turmeric, ginger, tulasi, neem, lemon, garlic, fenugreek etc. There are many bioactive compounds that have been derived from the plants possess anti-cancer activity. In United States about 50_60% of cancer has been treated using natural agents derived from plants as a complementary and as a alternative medicine along with traditional therapeutic regimen such as chemotherapy (Gutheil *et al*., 2011). In past, only the more developed countries were affected by the cancer but now the incidence of various forms of cancer is now rapidly rising worldwide. Meanwhile, there is no efficient medicine to treat cancer, because of side effects and multi-drug resistant strains of a number of pathogens. These draw backs leads to search plant-derived drugs that can reduce the side effects and also improves the health of patients. Research in phytochemistry has produced remarkably a diverse array of over 1,39,000 bio derived drugs (Cragg *et al*., 1996).

Nanoparticle are alternate sources to enhance the efficiency of cancer therapies. Because of its delivery and nil side effects, nanoparticles gained more attention in recent times towards treatment of various diseases. SPIONS are biocompatible nanoparticles mostly applied for drug delivery applications (Morteza Mahmoudi *et al*., 2011).

The pharmacologically significant compounds are present in almost parts of the body of medicinal plants (James Samuel Doughari). Here in this study, we took *Annona squamosa, a* plant with antibacterial activity (Padhi *et al*., 2011) and antidiabetic activity (Ranveer *et al*., 2012). Oral administration at a dose of 500 mg/kg body weight and 300 mg/ kg body weight respectively prevented the tumour formation and declined lipid peroxidation by enhancing the antioxidant mechanism (Suresh *et al*., 2006). Both extracts caused toxicity on tumour cells and downregulation of antiapoptotic genes (Khar *et al*., 2005).

On the basis of its traditional use leaf of *Annona squamosa* leaf was chosen to investigate its anticancer activity by fabricating the leaf extract with iron oxide against skin and liver cancer cell lines.

MATERIALS AND METHODS

Chemicals

The chemicals were purchased from Sigma, USA. All other chemicals used are of analytical grade.

Collection of Sample and preparation of extraction

Fresh leaves of *Annona squamosa* plant was collected in and around the region of Pallavaram, Chennai and authenticated by Plant Anatomy Research Centre,

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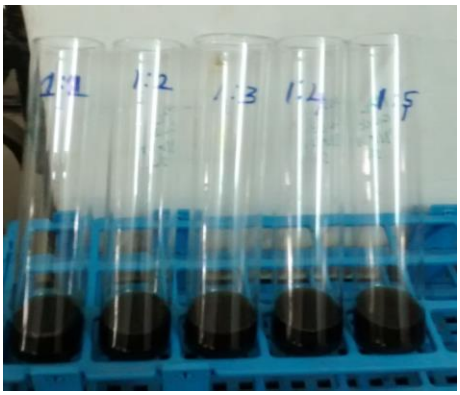


Figure 1: Visible colour change of Annona squamosa leaf extract

Table 1: Absorbance of the synthesized iron oxide nanoparticles

Sample volume ml	Absorbance nm
1:1	0.7926
1:2	0.8531
1:3	0.9151
1:4	3
1:5	3

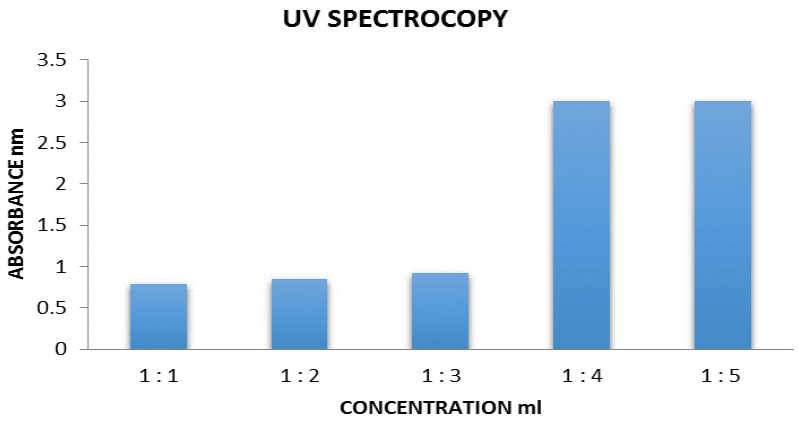
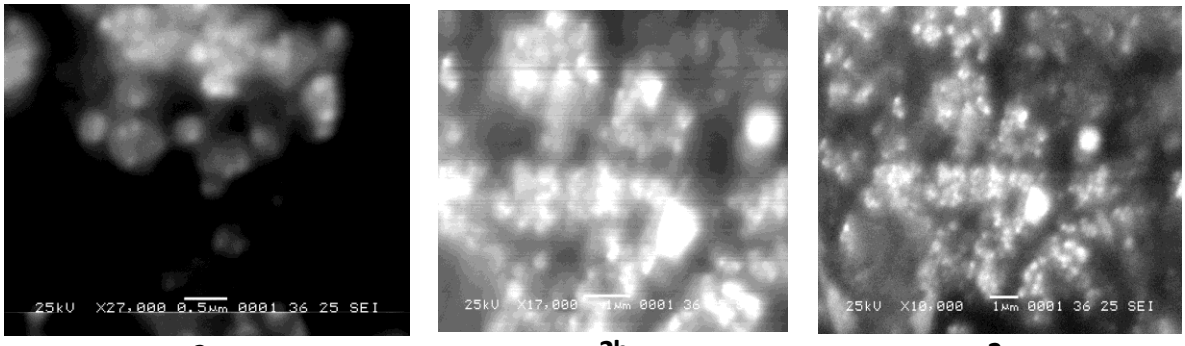


Figure 2: Absorbance obtained by UV-spectroscopy on various concentration gradient of the sample



2a **2b** **2c**

Figure 3: a: Typical electron energies are 1-25 kV with a spot size of 0.5nm; b: Typical electron energies are 1-17 kV with a spot size of 0001nm; c: Typical electron energies are 1-17 kV with a spot size of 1nm.

Chennai, India (PARC/2009/456). 70% hydroethanolic extract of the plant was prepared and stored after lyophilization for further analysis.

Synthesis & Analysis of Iron Oxide Nanoparticles

Iron oxide nanoparticles were synthesised by co-precipitation method with slight modifications (Anastasia *et al.*, 2016). UV-Visible Spectroscopy (Paul Mulvaney 1996) was performed to know SPR, SEM (Keum *et al.*, 2011) was performed to verify the uniformity and FTIR analysis for functional groups.

Cell Lines and Culture

HepG2 and human melanoma cell lines (A375) were used to perform cytotoxicity assay (Mosmann 1983).

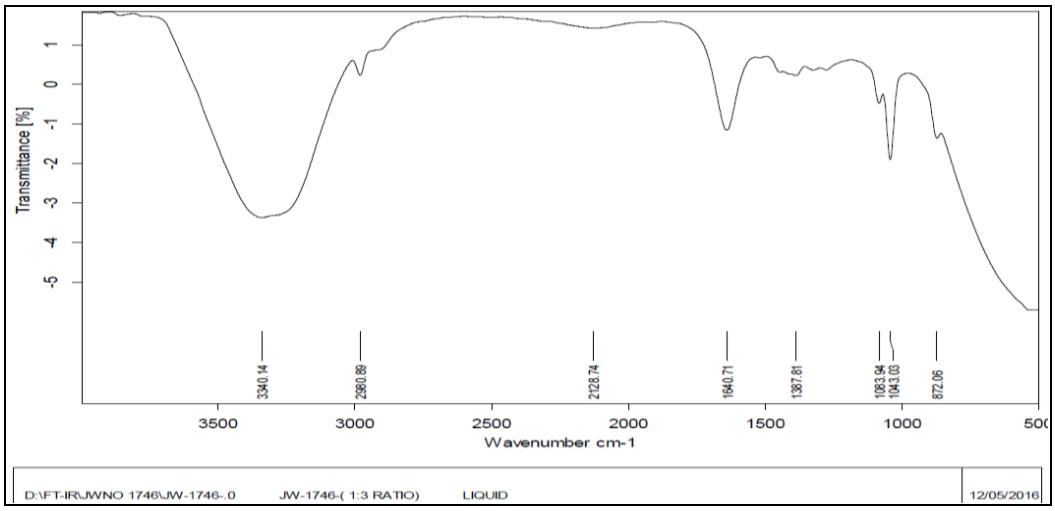


Figure 4: Representation of wave number cm-1 obtained by FT-IR analysis of the sample

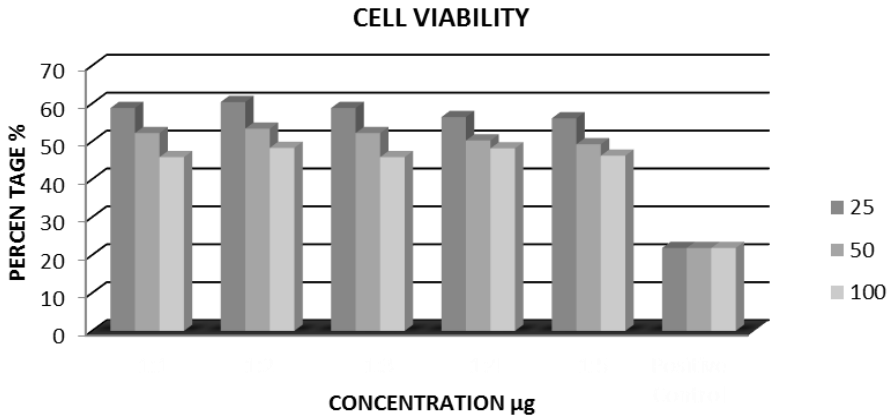


Figure 5: Percentage of cell viability by iron oxide nanoparticles on HepG2 Cell line

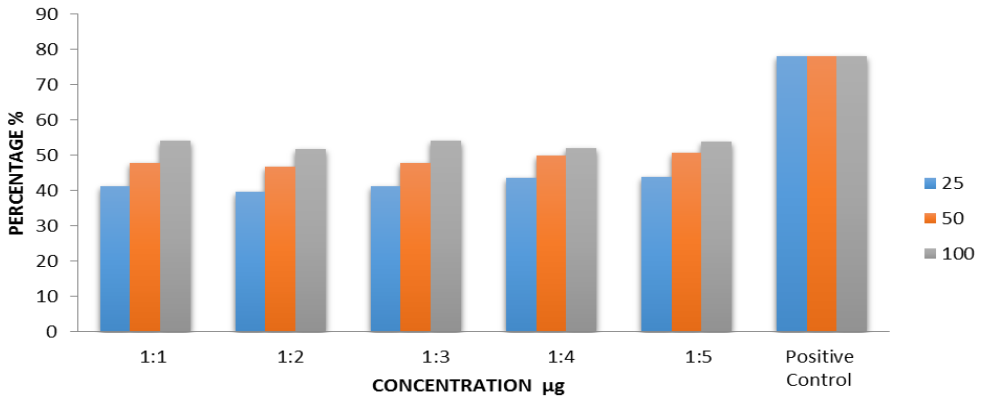


Figure 6: Percentage of Cell Cytotoxicity by Synthesized Iron Oxide Nanoparticles against HepG2 Cell Line

RESULT AND DISCUSSION

Due to efficient biomedical and environmental application of ironoxide nanoparticle researchers were looking into use of iron oxide nanoparticles for synthesis of drug with medicinal plants.

During synthesis of iron oxide nanoparticles using *Annona squamosa* leaf extract, a visible colour change from yellow colour into greenish black was observed. The intensity of colour increased with time and dosage of plant extract indicates the more growth of nanoparticles (Toshima *et al*., 1998).

Synthesis of Iron Oxide Nanoparticles

The addition of $FeCl_3$ to *Annona squamosa* leaves extract results in reduction reaction when the conversion of Fe^{3+} to Fe_3O_4 takes place. Initially, the C=O of the aldehyde group in *Annona squamosa* leaf extract chelated with Fe^{3+} ions to form ferric protein chains HO^- ...Fe^{3+} bonds and as result in the formation of suspended ferric hydroxide $Fe(OH)^3$. Subsequently, ferric hydroxide in a core is dehydrated ($-H_2O$) to form a black coloured magnetite (Fe_3O_4) nanoparticle as crystal. The protein chain in leaf extract may be covered on the

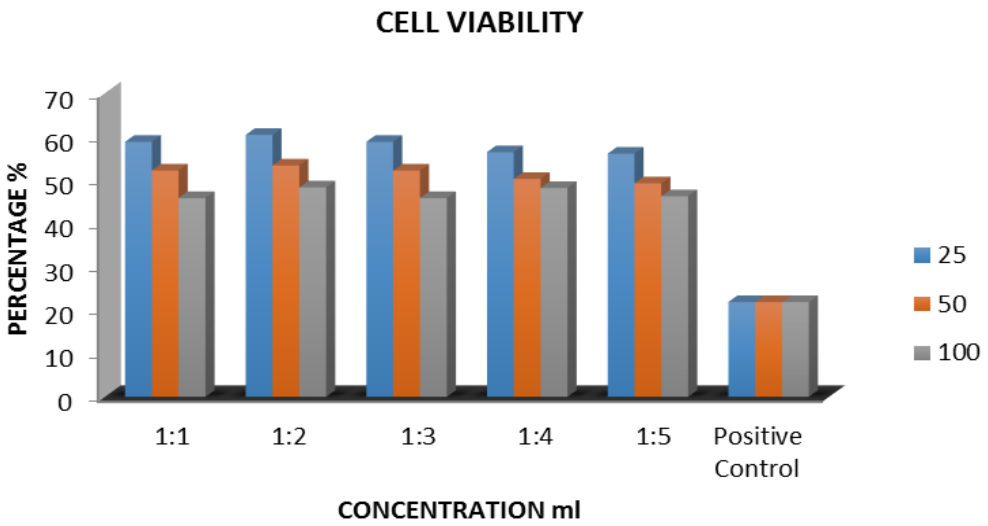


Figure 7: Percentage of cell viability by synthesised iron oxide nanoparticles in A375 cell line

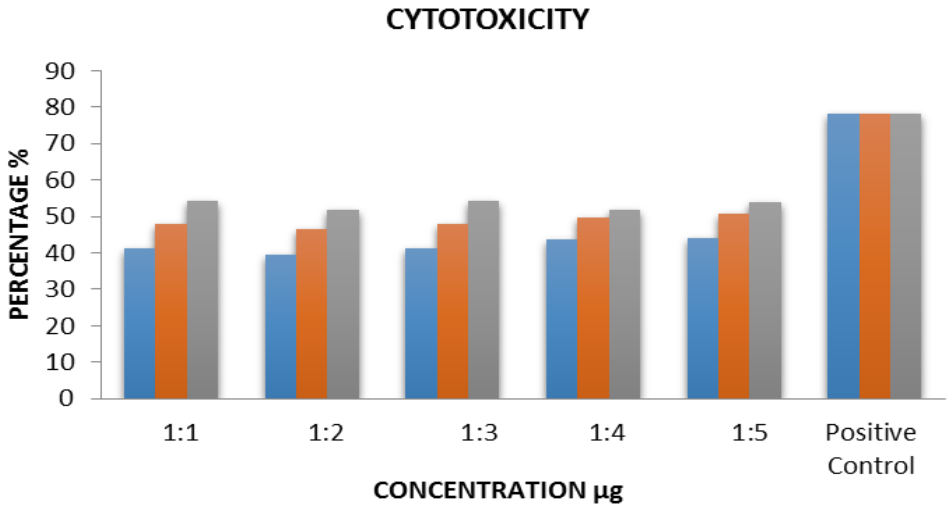


Figure 8: Percentage of cell cytotoxicity by synthesised iron oxide nanoparticles in A375 cell line

Fe_3O_4 surface through chelation of COO- ...Fe^{3+}

UV-Visible Spectroscopy

The surface plasmon resonances (SPR) of synthesized iron oxide nanoparticles have been studied by UV-Vis spectrophotometer. After the addition the *Annona squamosa* leaf extract into the aqueous solution of $FeCl_3$, the solution was filled in glass cuvette of path length 10mm and UV-Vis spectral analysis has been done in the range of 300 to 700 nm. DI water was used as a blank.

Various concentration of metal ions (1:1 – 1:5) solutions were mixed with the Annona squamosa leaves extract. After few minutes there was a colour change from black to blackish in the solution. This colour variation was due to concentration of metal ions and volume of the extract indicating the formation of iron oxides nanoparticles.

SEM Analysis

As Shown in Figure 2a-2c Fe_2O_3 nanoparticles grafted with *Annona squamosa* with a small spot by SEM analysis. Figure 2a- 2c shows the aggregation of the particles and small grains are present at the surface. Figure.2a shows that the particles are agglomerated with irregular distribution and morphology. The magnification at 25k x17000 in Figure2b, the Fe_3O_4 nanoparticles showed uniformly distributed small spherical shaped particles. The magnification at 25kx 10,000 (Figure2c) under the same conditions, which showed that large number of homogeneous nanocapsule like morphology of iron oxide nanoparticles.

Fourier Transform Infrared Spectrophotometer (FTIR)

Thus, the cytotoxic activity of the synthesised iron oxide nanoparticles was found to be dose-dependent and hence it was suggested that the cytotoxic activity may be due to the synthesised iron oxide nanoparticles from plant extract rather than ferrous sulphate.

2 shows the FT-IR spectrum of *Annona squamosa* leaf extract. From the Figure, the peak values are compared with reference ranges and found that alcohol, phenolic, amino, methyl, ketones, aldehydes and carboxylic acids were present. The absorbance band at 872 cm^{-1} represents oxidised iron oxide on the surface.

Cytotoxicity Assay

The effect of iron oxide nanoparticle on HepG2 cell line and Melanoma cell line and the cytotoxic activity were represented in Figure 4 and 6. The changes in cell viability indicated due to Fe3O4 NPs on various cancer cell lines was shown in Figure 3 and 5. No toxic*i*ty was seen in the normal liver cell line.

Thus, the cytotoxic activity of the synthesised iron oxide nanoparticles was found to be dose-dependent and hence it was suggested that the cytotoxic activity may be due to the synthesised iron oxide nanoparticles from plant extract rather than ferrous sulphate.

CONCLUSION

The synthesized iron oxide nanoparticles showed potential anticancer activity against cancer cells. Hence, it can be concluded that the iron oxide nanoparticles are powerful anticancer agents with therapeutic applications.

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